(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 221 700 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 86307922.4

(22) Date of filing: 14.10.86

(51) Int. Cl.⁴: A61K 47/00 , A61K 37/547

(30) Priority: 28.10.85 US 791836

(43) Date of publication of application:
13.05.87 Bulletin 87/20

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

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(54) A thrombin preparation.

(57) A thrombin preparation which has enhanced stability is disclosed. In addition to the thrombin, the preparation comprises a buffer in an amount sufficient to stabilise the thrombin and to buffer the preparation at a pH in the range of from 5.0 to 8.0. The preparation may also include saline and a polyol/polyalkylene polyol.

A method for making the preparation is also disclosed.

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A THROMBIN PREPARATION

Thrombin, a proteolytic enzyme, is essential for hemostasis. It is a principal reagent in the formation of blood clots via fibrin production. Due to its effectiveness as a clotting aid, thrombin and its preparations are useful during surgical procedures to control bleeding. While dry thrombin is available, liquid preparations are generally preferred due to handling and time considerations.

Until now, no highly stable liquid thrombin preparation which is both storage stable and ready for use during surgery has been available. This is because thrombin, when dissolved in water or saline alone, rapidly loses its activity due to denaturation and autolysis of the thrombin protein.

It has been discovered that sterile, storage-stable thrombin preparations can be prepared by adding to thrombin, in a suitable medium, a stabilizing quantity of a buffer. Optionally, saline and one or more polyol stabilizers can also be employed.

In a preferred embodiment, a solution containing 1000 U/ml (units per millilitre) Parke-Davis thrombin in 0.9% NaCl solution containing 25% (w/v) glycerol and 0.05 M sodium acetate buffer, pH 5.0, was prepared. This solution, after storage at room temperature for 39 days, had a clotting time of 14 seconds when measured on a fibrometer, which represents a retention of 70% of its original activity. A freshly prepared solution of the same composition had a clotting time of 10 seconds. The "unit" is a standard amount recognised in the art and relates to the quantity of blood which is clotted the by preparation.

The thrombin preparations and methods of the invention have several advantages over conventional preparations and methods for assisting in blood clotting.

Unlike powdered preparations, the use of the preparation of the instant invention requires no reconstitution before use. Thus, measuring, mixing, sterilizing, etc. of one or more components or containers are not necessary. The preparations of the present invention can be used with only minimal sterilization.

Furthermore, the stability of the thrombin containing preparations is so high that the need for stock inventories and/or rotation of products is largely eliminated. Unlike most saline-or water-solutions of thrombin, which are stable for only about 1 week at 4°C, the instant preparations are designed to be stable at normal refrigeration temperatures (i.e., about 4°C) for 6 months or more.

It is known that high concentrations or glycerol, sucrose, and other polyols can stabilize proteins in solution. In the case of thrombin, it is known that a glycerol concentration of 67% can greatly stabilize a 1000 u/ml thrombin solution. However, the use of high glycerol concentrations is not practical in the large scale manufacture of a sterile thrombin solution because of the high viscosity of such a preparation.

According to a first aspect of the present invention, there is provided a preparation comprising thrombin and having an enhanced stability,
characterized in that the preparation also includes a buffer present in an amount sufficient to stabilise the thrombin and to buffer the preparation at a pH in the range of from 5.0 to 8.0.

According to a second aspect of the present invention there is provided a haemostat for use as a wound dressing, the hemostat comprising a preparation in accordance with the first aspect of the present invention and a substrate.

According to a third aspect of the present invention, there is provided a method for manufacturing a thrombin preparation having enhanced stability, said process comprising, combining thrombin with a quantity of a buffer sufficient to stabilise the thrombin and to buffer the preparation at a pH in the range of from 5.0 to 8.0.

The preparations made in accordance with the present invention must contain, in a liquid medium, thrombin and one or more buffers. They may contain saline, polyols and other substances conventionally employed in thrombin preparations.

While the term "preparations" is employed, it should be noted that all types of formulations in which thrombin is substantially solubilized, or in a highly dispersed form, and is present in combination with one or more buffers are contemplated.

In general liquid preparations are preferred, and solutions in which the thrombin is solubilized are highly preferred. When a liquid formulation is made, it is generally preferred that the or each solvent or other diluent employed be sufficiently miscible with thrombin such that production standards, e.g., uniformity of thrombin concentration from batch to batch, can be readily met.

The thrombin employed is commercially available. A preferred thrombin is THROMBOSTAT^{R} powder, marketed by PARKE-DAVIS. It contains, in addition to thrombin, 30% (w/w) glycerine, 5.3% $CaCl_2 2H_2O$ and 5.5% NaCl. THROMBOSTAT^{R} is supplied in vials containing 5000 units, 10000 units and 20000 units.

Thrombin is known to be soluble in physiological saline -i.e., a solution containing 0.9% NaCl in water. However, saline solutions with higher concentrations are contemplated as useful herein. Furthermore, the replacement of all or part of the NaCl in such solutions with one or more other suitable salts is contemplated.

Water is a preferred medium for the preparations of the invention. However, the use of one or more other diluents which do not adversely affect the solubility and/or stability of thrombin in the subject preparations can be employed.

One such diluent is glycerol. Glycerol and other polyols, such as polyaklylene glycols and preferably polyethylene glycols, are typical ingredients in many commercial thrombin-based products. Other useful polyols include mannitol, sorbitol, sucrose, glucose, and the like. Mixtures are operable.

The buffer employed in the preparations of the present invention is prepared in the final aqueous formulation, and before the formulation is mixed with the thrombin. For instance, in order to prepare a formulation buffered at pH 5.3 with acetate and containing 25% glycerol in 0.9% NaCl, the glycerol-containing saline is first prepared, and the required amount of acetic acid is added. The pH is then adjusted to 5.3 with a strong sodium hydroxide solution.

Alternatively, the buffer can be prepared by adding sodium acetate and adjusting the pH with a strong acid, or, thirdly, the buffer can be prepared by adding acetic acid and sodium acetate in a mole ratio calculated to produce the desired pH.

Suitable buffer systems are those whose aqueous solutions will maintain the pH of the final thrombin solution between 5.0 and 8.0, with a preferred pH range of 5.0 to 6.0 and a highly preferred range of 5.3 to 5.7. Useful buffer systems include acetate, succinate, bicarbonate, imidazole, TRIS, and zwitterionic buffers described by N.E. Good and S. Izawa, in Methods in Enzymol, 24, Part B, 53 (1972): and W.F. Ferguson, K.I. Braunschweiger, W.R. Braunschweiger, J.R. Smith, J. McCormick, C.C. Wasmann, N.P. Jarvis, D.H. Bell and N.E. Good in Anal. Biochem, 104, 300 (1980).

Suitable reagents for use in the buffer systems include MES, ACES, BES, MOPS, TES, HEPES and the like. Phosphate can only be used when calcium ion is absent or in the presence of EDTA. Mixtures of such reagents can be employed.

Useful buffer systems also include acetic acid-sodium acetate, acetic acid-potassium acetate, bicarbonate, succinate, imidazole, and TRIS salts. Sodium acetate/acetic acid is a preferred reagent. Mixtures are operable.

The buffers will be present in the buffer solution, along with water and/or other suitable diluent, at total concentrations of from 0.01M to 1M, preferably from 0.02 to 0.2M.

The use of various other conventional additives, e.g., antioxidants, colourants, surfactants, and the like, is also contemplated. Lysine and/or other amino acids may be employed as optional ingredients.

In general, the concentration ranges for the ingredients discussed above will be within the limits set out in Table 1. Percentages are based on total composition weight.

Table 1

Weight Percentage

Ingredient	Broad	Preferred	Highly Preferred
Thrombin units/ml	10-10000	50-5000	100-1000
Buffer solution (M)	0.01-1.0	0.02-0.2	0.05-0.10
Diluent/Solvent	-	-	-
Polyol	0-50	10-40	10-25
NaCl	0-5	0.9-2.7	0.9-1.8

Hemostatic materials such as GELFOAM, SURGICAL, and AVICEL which are presently used alone or in combination with thrombin powder or thrombin in saline, can be effectively used with the stabilized thrombin solution of the present invention. The stabilized solution can be absorbed onto the hemostatic agent and the wet pad can be packaged in a sterile manner.

Antimicrobial or antibiotic agents can be incorporated into such pads, especially for use on burn patients, where prevention of infection is critical. In addition, surfactants and salts other than NaCl can be employed. When one or more of such additives are present, their concentrations are generally within the ranges set out in Table II.

Table II

Weight Percentage

Additive	Broad	Preferred	Highly Preferred
Surfactants	0-2	0-0.5	0-0.2
Antioxidants	0-1	0-0.2	0-0.1
Antimicrobials	0-1	0-0.2	0-0.1
Other Additives e.g. salts	0-5	0-3	0-1

One type of bandage suitable in the preparation of coagulants in accordance with the invention is set forth in U.S. Patent 4,363,319.

Production of Thrombin Preparations

The thrombin formulations made in accordance with the present invention are made by conventional processing techniques. The use of particular devices for mixing, adding, etc. should not be regarded as a limitation.

The order of addition of the ingredients is believed to be critical, and it is generally preferred that all the ingredients except thrombin be mixed first, and the pH adjusted to 5.0, before addition of thrombin-containing powder.

EXAMPLES

The following is an example of a preferred formulation. Sodium chloride, 0.9g, and glycerol, 25.0g, are dissolved in approximately 75ml of distilled, deionized water. To this solution is added 0.29 ml of glacial acetic acid. The mixture is diluted to approximately 90ml and the pH is adjusted to 5.0 with 5N sodium hydroxide. The volume of the solution is then brought to exactly 100ml by addition of distilled, deionized water. To prepare, for example, a 1000 unit/ml thrombin solution, 20ml of buffered formula are added to a vial containing 20000 units of thrombin in THROMBOSTAT powder, or 10ml of buffered formula are added to a vial containing 10000 units of thrombin, or 5.0ml of buffered formula are added to 5000 units of thrombin. The thrombin solution is shaken gently or otherwise agitated to dissolve the THROMBOSTAT powder, and the solution is stored at 4°C until ready for use.

Table III below shows levels of thrombin activity remaining in thrombin solutions after storage at various temperatures. It is clear that the presence of a buffer significantly enhances the storage stability of thrombin preparations.

Table III

Percentage of Original Thrombin Activity After Storage at Various Temperatures

Composition	Temp (Storage Time) 37°C (11 days)	Temp (Storage Time) 25°C (41 days)
THROMBIN (1500 units/ml 0.9% saline pH 6.80	0	0
THROMBIN (1500 units/ml 25% (v/v) glycerol 0.9% NaCl pH 6.80	26	15
THROMBIN (1500 units/ml 25% w/w glycerol 0.9% NaCl, 0.05M acetate buffer pH 5.13	68	71

The thrombin activity levels were determined by measurement of clotting time on a BBL fibrometer. The source of fibrinogen was pooled human plasma diluted 1:1 with 0.9% saline. The thrombin solution was diluted 200-fold with 0.5% polyethylene glycol 8000 in imidazole buffered saline. Into a coagulation cup was added 0.2ml of diluted plasma. This was kept at 37°C for 3 minutes, and to this solution was added 0.1ml of diluted thrombin solution, which had also been kept at 37°C for 3 minutes. Clotting time was determined directly from the fibrometer reading. The number of thrombin units/ml remaining was determined from a standard curve of thrombin concentration vs. clotting time.

The data Table IV show that, while unbuffered solutions containing glycerol in saline provide some stability to low levels of solubilized thrombin, high levels of solubilized thrombin cannot be sterilized. In contract, the degrees of stabilization of both low and high concentrations of thrombin in the buffered composition of the present invention are approximately the same, and are much greater than that provided by glycerol alone.

TABLE IV

Percentage of original activity of various concentrations of solubilized thrombin after storage at 37°C for 2 weeks

Composition	THROMBIN CONCENTRATION (UNITS/ML) 250	500	1000
THROMBIN 25% (w/w) glycerol 0.9% NaCl pH 6.8	50	30-40	<30
THROMBIN 25% (w/w) glycerol 0.9% NaCl 0.05M acetate buffer pH 5.1	70-80	70-80	70-80

Claims

Claims for the following Contracting States : BE, CH, DE, FR, GB, IT, LI, LU, NL and SE

1. A preparation comprising thrombin and having an enhanced stability, characterized in that the preparation also includes a buffer present in an amount sufficient to stabilise the thrombin and to buffer the preparation at a pH in the range of from 5.0 to 8.0.

2. A preparation according to Claim 7, which is buffered at a pH in the range of from 5.0 to 6.0, preferably at a pH of about 5.3.

3. A preparation according to Claim 1 to 2, further comprising saline and at least one polyhydroxy stabilizer.

4. A preparation according to Claim 3, wherein the stabilizer is chosen from C_{3-12} polyols, polyethylene polyols having molecular weights in the range of from 200 to 8000 and mixtures thereof.

5. A preparation according to any preceding Claim, wherein the buffer is an acetate buffer.

6. A hemeostat, for use as a wound dressing, comprising a preparation according to any preceding claim and a substrate.

7. A method for manufacturing a thrombin preparation having enhanced stability, which method comprises, combining thrombin with a quantity of a buffer sufficient to stabilise the thrombin and to buffer the preparation at a pH in the range of from 5.0 to 8.0.

8. A method according to Claim 7, wherein the preparation is buffered at a pH in the range of from 5.0 to 6.0, preferably at a pH of about 5.3.

Claims for the following Contracting State : AT, ES and GR

1. A method for manufacturing a thrombin preparation having enhanced stability, which method comprises, combining thrombin with a quantity of a buffer sufficient to stabilise the thrombin and to buffer the preparation at a pH in the range of from 5.0 to 8.0.

2. A method according [redacted] laim 7, wherein the preparation is buffe[redacted] a pH in the range of from 5.0 to 6.0, preferably at a pH of about 5.3.

3. A method according to Claim 1 to 2, comprising further combining the thrombin buffer with saline and at least one polyhydroxy stabilizer.

4. A method according to Claim 3, wherein the stabilizer is chosen from C_{3-12} polyols, polyethylene polyols having molecular weights in the range of from 200 to 8000 and mixtures thereof.

5. A method according to any preceding Claim, wherein the buffer is an acetate buffer.

6. A hemeostat, for use as a wound dressing, comprising a preparation prepared by a method as claimed in any preceding claim and a substrate.